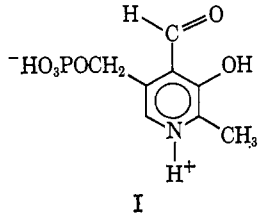
# Equilibrium Studies Involving Schiff Base Complexes. The Zinc(II)–Pyridoxal Phosphate–Glycine and –α-Alanine Systems[1]

**W. L. Felty, C. G. Ekstrom, and D. L. Leussing**

*Contribution from the Department of Chemistry, The Ohio State University, Columbus, Ohio 43210.* *Received August 7, 1969*

**Abstract:** Equilibrium constants describing the protonation equilibria and the formation of binary and ternary species between Zn(II), pyridoxal phosphate, and either glycine or α-alanine have been determined from pH titration data. The stabilities of the unprotonated Schiff bases were evaluated spectrophotometrically. Mixed complexes of the type $Zn(PLP\cdot L)^{2-}$, $Zn(PLP\cdot L)(L)^{3-}$, and $Zn(PLP\cdot L)_2^{6-}$ (PLP = pyridoxal phosphate, L = alaninate or glycinate), plus four mono- and diprotonated forms, are formed in solution to a significant extent. The stability constants and compositions of the ternary complexes indicate that the ligands are condensed as the Schiff base. Stabilities of the mono Schiff base complexes containing either glycine or alanine are essentially equal, but with the bis species the alanine complex is slighty weaker. The protonation scheme of the Schiff base and the Schiff base complexes has been elucidated and the basicity of the pyridine nitrogen in different related species is compared. The phosphate group of PLP appears to interact to some extent with a complexed metal ion.

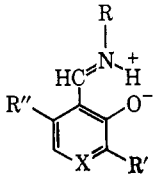
Pyridoxal 5′-phosphate (PLP, I) is known to be the necessary cofactor in enzymatic transamination reactions.[2] Numerous studies, summarized elsewhere,[2,3] have demonstrated that nonenzymatic trans-



I

amination is catalyzed by certain metal ions in model systems containing amino acid, carbonyl compound, and metal ion. The mechanism in both enzymatic and nonenzymatic cases is considered to involve the formation of intermediate Schiff bases which then undergo reaction under the influence of enzyme or metal ion.[4]

A series of investigations on the behavior of Schiff base complexes in aqueous media is currently underway in these laboratories. Utilizing pH titration experiments supplemented by spectrophotometric data, equilibrium studies have been carried out on systems of increasing complexity as the carbonyl compound used was varied from aliphatic acids such as pyruvic[5,6] and glyoxylic,[7] where a large difference exists between the $pK_a$ for the carboxylic acid and the $pK_{2a}$ for the amino acid, through salicylaldehyde,[8,9] where the $pK_a$ differences are much smaller, to pyridoxal (PL),[10] where protonation of the pyridine nitrogen causes an additional series of protonated complexes to be formed. The aromatic carbonyl systems also have the complication that proton-stabilized, noncomplexed Schiff



bases may be formed to an appreciable degree and must be taken into account in the species distribution.

In spite of the fact that pyridoxal phosphate (PLP) is the active cofactor in enzymatic reactions, PL has been employed considerably more often than PLP in studies on model systems. To a large extent the reason for this is to avoid the additional complication arising from PLP phosphate protonation equilibria. On the other hand, PL systems undergo hemiacetal formation between the free alcohol and aldehyde groups. This reaction also complicates the equilibria and drastically slows reaction rates.

Schiff base formation between PLP and various amino acids has been demonstrated spectrophotometrically.[11–16] Because the pH dependence in these former studies was not adequately investigated only pH-dependent conditional constants[17] can be validly calculated from the data.[11,13,16] Stable PLP Schiff base complexes, also, have been shown to form in solution[11,13–15] but again the data at best yield only conditional constants. A thorough investigation of PLP–amino acid–metal ion equilibria has heretofore not appeared in the literature.

We have now extended the methods used in the previous studies to describe the complicated distribution of species found in aqueous solutions containing the

(1) The support of this work by the National Science Foundation is gratefully acknowledged.

(2) E. E. Snell, P. M. Fasella, A. Braunstein, and A. Rossi-Fanelli, Ed., "Chemical and Biological Aspects of Pyridoxal Catalysis," The Macmillan Co., New York, N. Y., 1963, p 1.

(3) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 2, W. A. Benjamin, Inc., New York, N. Y., 1966, Chapter 8.

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biologically important aldehyde, PLP, with Zn(II) and either glycine or alanine. The results reported here are a prerequisite to an understanding of kinetic studies presently in progress and are of interest in increasing our understanding of the interactions in systems of this type.

## Experimental Section

Glycine and DL-α-alanine were obtained from Sigma Chemical Co. and used without further purification, since analysis by formal titration[18] yielded purities of 100.0 ± 0.2%.

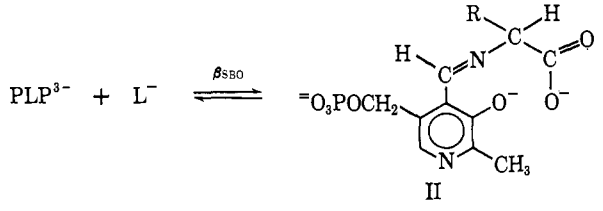
Stock solutions of $ZnCl_2$ were prepared and standardized using accepted procedures.

PLP, obtained from Nutritional Biochemical Corp. and Sigma Chemical Co., was used without additional purification. The purity was determined by titration with standard NaOH to the third equivalence point and found to be in the range 98–99% for different lots. Stock solutions of 0.02 *M* PLP were freshly prepared by dissolving weighed quantities of PLP in aqueous solutions of KCl.

Titrations were carried out on solutions initially containing 0.01 *M* PLP, varying concentrations of $ZnCl_2$ (0, 0.001, 0.002, 0.004, or 0.005 *M*) and amino acid (0, 0.005, 0.01, 0.02 *M*), and sufficient KCl to bring the ionic strength to 0.5. Standard, carbonate-free 0.2 *M* NaOH, 0.3 *M* KCl was used as titrant. The pH was recorded as a function of time after each addition of titrant to determine equilibration times, which were of the order of 5–15 min in the slower ternary systems. Titrated solutions were thermostated at 25.0 ± 0.1° and maintained under a blanket of nitrogen. At least three titrations were run for the determination of each equilibrium constant.

pH measurements were made using a Model 401 Orion digital pH meter with either a Corning No. 476022 glass electrode and Radiometer Type K4312 calomel electrode or a No. 476051 Corning combination electrode. The pH meter was standardized with NBS tartrate and phosphate buffers. Spectrophotometric measurements were made on a Cary 14 spectrophotometer using thermostated cell holders.

The formation constant, $\beta_{SBO}$, for the unprotonated Schiff base




II

could not be reliably determined by potentiometric titration and therefore was evaluated spectrophotometrically.[8,10] A series of solutions was prepared, each containing 0.0014 *M* total PLP, 0.02 *M* NaOH, varying concentrations (0 to 0.5 *M*) of NaG or NaA ($G^-$ = glycinate, $A^-$ = alaninate), and sufficient KCl to adjust the ionic strength to 0.50. Schiff base formation was evidenced by a decrease in the $PLP^{3-}$ band at 388 mμ ($\epsilon_{388}$ 6.82 × $10^3$ $M^{-1}$ $cm^{-1}$), an isosbestic point at 360 mμ, and increased absorbance in the region 359–280 mμ as the amino acid concentration was increased. Schiff base formation under these conditions is rapid and appears to have been completed by the time the mixed solutions were placed in the spectrophotometer cell and measurements begun (3–5 min). However, slow changes in the spectra were observed over a period of time due to tautomerization, since maxima emerged corresponding to those of pyridoxamine phosphate.[15,19] The absorbance at 388 mμ was monitored and extrapolated to zero time, the small correction amounting to no more than 3% of $A_{388}$. The formation constants and the extinction coefficients of the unprotonated Schiff bases are $\beta_{SBO}$ = 4.6, $\epsilon_{SB,388}$ 1.4 × $10^3$ $M^{-1}$ $cm^{-1}$ for glycine and $\beta_{SBO}$ = 3.4, $\epsilon_{SB,388}$ 1.7 × $10^3$ $M^{-1}$ $cm^{-1}$ for alanine.

All other constants for PLP-containing species were obtained by a least-squares fit of calculated pH titration curves to the observed data as described earlier.[10] The titration curves for the Schiff base systems are shown in Figure 1. Similar data for the binary systems were also obtained. The amino acids form a series of binary com-

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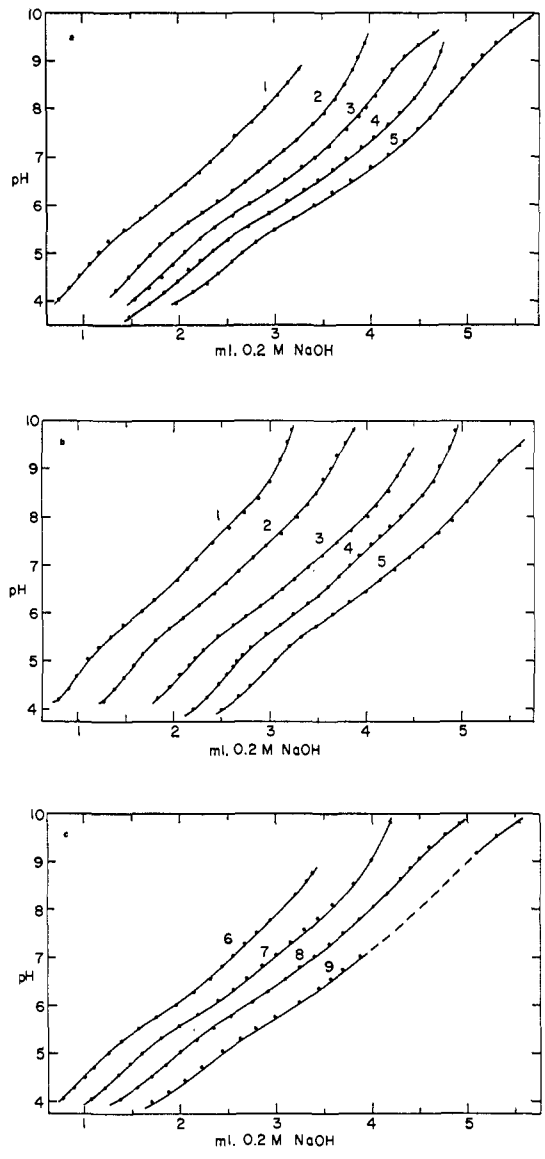



Figure 1. Titration of zinc chloride, pyridoxal phosphate, and amino acid solutions; initial volumes, 20 ml. The solid lines are theoretical curves calculated using the results in the text.

(a) Glycine: titrant, 0.1999 *M* NaOH, 0.3 *M* KCl. Initial concentrations (*M*) are given below.

| Titration no. | $ZnCl_2$ | $H_3PLP$ | HG | HCl |
|---|---|---|---|---|
| 1 | 0.002000 | 0.00995 | 0.005000 | 0.000040 |
| 2 | 0.002000 | 0.00995 | 0.01000 | 0.000040 |
| 3 | 0.002000 | 0.01000 | 0.02000 | 0.000040 |
| 4 | 0.004000 | 0.00995 | 0.01000 | 0.000080 |
| 5 | 0.004000 | 0.00995 | 0.02000 | 0.000080 |

To facilitate presentation, curves 2, 3, 4, and 5 are shifted to the right by 0.5, 0.8, 1.0, and 1.25 ml, respectively.

(b), (c) Alanine: titrant, 0.1983 *M* NaOH, 0.3 *M* KCl. Initial concentrations (*M*) are given below.

| Titration no. | $ZnCl_2$ | $H_3PLP$ | HA | HCl |
|---|---|---|---|---|
| 1 | 0.001000 | 0.00978 | 0.005004 | 0.000017 |
| 2 | 0.001000 | 0.00978 | 0.01006 | 0.000017 |
| 3 | 0.002000 | 0.01002 | 0.01005 | 0.000034 |
| 4 | 0.002001 | 0.01002 | 0.005004 | 0.000034 |
| 5 | 0.002001 | 0.01003 | 0.02012 | 0.000034 |
| 6 | 0.004002 | 0.01003 | 0.005004 | 0.000067 |
| 7 | 0.004002 | 0.01003 | 0.01006 | 0.000067 |
| 8 | 0.004002 | 0.01003 | 0.02012 | 0.000067 |
| 9 | 0.005002 | 0.01001 | 0.02012 | 0.000082 |

To facilitate presentation, curves 2, 3, 4, and 5 are shifted to the right by 0.5, 1.0, 1.5, and 1.8 ml, respectively, and curves 7, 8, and 9, by 0.3, 0.6, and 1.0 ml, respectively. In titration 9 turbidity was observed over the dashed portion of the curve.

**Table I.** Equilibrium Constants for Zn(II)–Pyridoxal Phosphate–Glycine and -α-Alanine Systems at 25°, $I$ = 0.5 (KCl)

A. Species Not Containing Zn(II)

| | Simple Protonation Constants[a] | | | |
|---|---|---|---|---|
| | $pK_{1a}$ | $pK_{2a}$ | $pK_{3a}$ | $pK_{4a}$ |
| PLP | 1.6 (ref 22) | 3.63 ± 0.02 | 5.98 ± 0.01 | 8.14 ± 0.02 |
| G[b] | 2.46 | 9.70 | | |
| A | 2.45 ± 0.01 | 9.81 ± 0.01 | | |

| | PLP Schiff Base Formation Constants[c] | | | | |
|---|---|---|---|---|---|
| L | Log $\beta_{SB0}$ | Log $\beta_{SB1}$ | Log $\beta_{SB2}$ | $pK_{4a}$[d] | $pK_{5a}$ |
| G | 0.67 | 12.55 ± 0.01 | 19.20 ± 0.03 | 6.65 | 11.88 |
| A | 0.53 | 12.30 ± 0.01 | 18.63 ± 0.08 | 6.33 | 11.77 |

B. Zn(II)-Containing Species

$$Zn^{2+} + iPLP^{3-} + jL^{-} + kH^{+} \overset{\beta_{ijk}}{\rightleftharpoons} Zn(PLP)_iL_jH_k^{2-3i-j+k}$$

$$\beta_{ijk} = \frac{(Zn(PLP)_iL_jH_k^{2-3i-j+k})}{(Zn^{2+})(PLP^{3-})^i(L^{-})^j a_H^k}$$

$$Zn(PLP)_iL_jH_k^{2-3i-j+k} + H^{+} \overset{K_{ijk}}{\rightleftharpoons} Zn(PLP)_iL_jH_{k+1}^{3-3i-j+k}$$

$$K_{ijk} = \frac{(Zn(PLP)_iL_jH_{k+1}^{3-3i-j+k})}{(Zn(PLP)_iL_jH_k^{2-3i-j+k})a_H}$$

| | Glycinate | Alaninate |
|---|---|---|
| **Log Binary Constants** | | |
| $\beta_{010}(Zn^{2+} + L^{-} \rightleftharpoons ZnL^{+})$ | 4.85 | 4.57 |
| $\beta_{020}(Zn^{2+} + 2L^{-} \rightleftharpoons ZnL_2)$ | 9.14 | 8.56 |
| $\beta_{030}(Zn^{2+} + 3L^{-} \rightleftharpoons ZnL_3^{-})$ | 11.81 | 10.59 |
| $\beta_{100}(Zn^{2+} + PLP^{3-} \rightleftharpoons Zn(PLP)^{-})$ | 3.6 | |
| $K_{101}(Zn(PLP)^{-} + H^{+} \rightleftharpoons Zn(PLP)H)$ | 6.3 | |
| $K_{102}(Zn(PLP)H + H^{+} \rightleftharpoons Zn(PLP)H_2^{+})$ | 5.6 | |
| **Log Ternary Constants** | | |
| $\beta_{110}(Zn^{2+} + PLP^{3-} + L^{-} \rightleftharpoons Zn(PLP\cdot L)^{2-})$ | 10.28 ± 0.17 | 10.26 ± 0.06 |
| $\beta_{120}(Zn^{2+} + PLP^{3-} + 2L^{-} \rightleftharpoons Zn(PLP\cdot L)(L)^{3-})$ | 13.79 ± 0.18 | 13.82 ± 0.11 |
| $\beta_{220}(Zn^{2+} + 2PLP^{3+} + 2L^{-} \rightleftharpoons Zn(PLP\cdot L)_2^{6-})$ | 18.05 ± 0.03 | 17.08 ± 0.04 |
| $K_{111}(Zn(PLP\cdot L)^{2-} + H^{+} \rightleftharpoons Zn(PLP\cdot L)H^{-})$ | 7.45 ± 0.17 | 7.40 ± 0.04 |
| $K_{112}(Zn(PLP\cdot L)H^{-} + H^{+} \rightleftharpoons Zn(PLP\cdot L)H_2)$ | 5.44 ± 0.03 | 5.45 ± 0.03 |
| $K_{221}(Zn(PLP\cdot L)_2^{6-} + H^{+} \rightleftharpoons Zn(PLP\cdot L)_2H^{5-})$ | 8.32 ± 0.03 | 8.60 ± 0.03 |
| $K_{222}(Zn(PLP\cdot L)_2H^{5-} + H^{+} \rightleftharpoons Zn(PLP\cdot L)_2H_2^{4-})$ | 6.94 ± 0.04 | 7.16 ± 0.06 |

[a] All proton constants are defined in terms of hydrogen ion activity, $a_H$, and molar concentrations of other species, $K_a = a_H(B)/(HB)$. G = glycine, A = alanine. [b] From ref 5. [c] $PLP^{3-} + L^{-} + iH^{+} \overset{\beta_{SBi}}{\rightleftharpoons} PLPLH_i^{i-4}$, $\beta_{SBi} = (PLPLH_i^{i-4})/(PLP^{3-})(L^{-})a_H^i$. [d] $pK_{4a} = \log\beta_{SB2} - \log\beta_{SB1}$, $pK_{5a} = \log\beta_{SB1} - \log\beta_{SB0}$.

plexes, $ZnL^{+}$, $ZnL_2^{0}$, $ZnL_3^{-}$, and pyridoxal phosphate was found to bind zinc in a 1:1 ratio, yielding $ZnPLP^{-}$, ZnPLPH, and $ZnPLPH_2^{+}$. The results obtained earlier[5-10] were used as guide in interpreting the data obtained for the PLP–Zn(II)–amino acid ternary systems. The species having the overall compositions (Schiff base complexes included)[20] $Zn(PLP)(L)^{2-}$, $Zn(PLP)(L)_2^{3-}$, and $Zn(PLP)_2(L)_2^{6-}$ were considered, as were also the mono- and diprotonated forms of $Zn(PLP)(L)^{2-}$ and $Zn(PLP)_2(L)_2^{6-}$, since protonation of both the pyridine nitrogen and the phosphate group of PLP was anticipated. Preliminary calculations using the pyridoxal constants[10] and the phosphate protonation constants as estimates indicated that tri- and higher protonated complexes were probably formed in negligible amounts (even for $Zn(PLP)_3(L)_2^{6-}$) and so these species were omitted from the calculations. No need to consider these species further arose as the data were analyzed. Similarly, levels of $Zn(PLP)(L)_2(H)_k^{k-3}$ ($k$ = 1, 2) were indicated by preliminary calculations to be low (< 3% of total Zn) so the protonation constants were assumed to be the same as those for $Zn(PLP)(L)^{2-}$. Thus, the formation constants for seven ternary species were determined from the titration curves shown in Figure 1. The constants are defined in Table I. Unlike the pyridoxal system,[10] it was not found possible to relate the ionization of protonated Schiff base complexes to only one or two separate $pK_a$ values by assuming a statistical relationship between protonated forms.

Excellent fits were obtained between experimental points and calculated curves as can be seen from Figure 1. Using the values given in Table I, the standard deviation of a point was 0.023 pH unit for glycine (total of 108 points) and 0.028 pH unit for alanine (total of 193 points). The uncertainties assigned to the ternary constants in Table I reflect the fit of theoretical and observed titration curves. Unknown systematic errors, such as ligand purities, could produce larger differences. The fact that two different systems yield similar values, as expected, supports the validity of the qualitative conclusions and gives credence to the quantitative results.

## Results and Discussion

The proton constants for PLP found in this work are in reasonable agreement with literature values considering slightly different conditions and methods of determination.[19,21,22] No attempt was made here to determine $pK_{1a}$, but the value of 1.6[22] was used to account for the small amount of $H_4PLP^{+}$ formed at low pH.

(20) It should be noted that the equilibrium data yield only the overall compositions and stabilities of the various species that are formed, $Zn_h(PLP)_i(L)_j(H)_k^{2h+k-3i-j}$. Evidence that the amine donors and carbonyl donors in a mixed complex ($i > 0$ and $j > 0$) are condensed as Schiff bases may be obtained from the values of the formation constants as well as from the spectral characteristics (see ref 5–10). In the present system, the Schiff base forms predominate in those species where $j \geq i$. When it is desired to emphasize the Schiff base character, the species are alternatively written $Zn(PLP\cdot L)_i(L)_{j-i}(H)_k$. Only mononuclear species ($h$ = 1) were observed in this work.

(21) N. Christensen, *J. Amer. Chem. Soc.*, **80**, 99 (1958).
(22) F. J. Anderson and A. E. Martell, *ibid.*, **86**, 715 (1964).

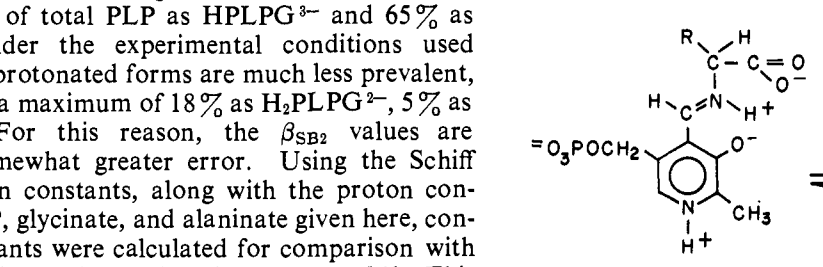
The presence of amino acid caused the PLP titration curve to be depressed to lower pH values in the region corresponding to the removal of the first and second protons. This indicates the formation of mono- and diprotonated Schiff bases. The overall formation constants, $\beta_{SBi}$, for the reactions $PLP^{3-} + L^- + iH^+ \rightleftharpoons PLP \cdot L \cdot H_i^{i-4}$ were then evaluated from data in this region. The results showed that the monoprotonated species are formed to a large extent, reaching a maximum of 85% of total PLP as $HPLPG^{3-}$ and 65% as $HPLPA^{3-}$ under the experimental conditions used here. The diprotonated forms are much less prevalent, amounting to a maximum of 18% as $H_2PLPG^{2-}$, 5% as $H_2PLPA^{2-}$. For this reason, the $\beta_{SB2}$ values are subject to somewhat greater error. Using the Schiff base formation constants, along with the proton constants for PLP, glycinate, and alaninate given here, conditional constants were calculated for comparison with those reported by Matsuo[13] and Lucas, *et al.*[11] This comparison is given in Table II. With the exception at high pH with glycine and at low pH with alanine the agreement is quite satisfactory considering the different conditions.

**Table II.** Comparison of Conditional Formation Constants for PLP–Amino Acid Schiff Bases

| pH | Log $K_{cond}$ Matsuo (ref 13)[a] | Lucas (ref 11) | This work |
|---|---|---|---|
| | Glycine | | |
| 5.5 | | 0.6 | 0.78 |
| 6.0 | | 1.2 | 1.15 |
| 6.5 | | 1.4 | 1.47 |
| 7.0 | | 1.5 | 1.80 |
| 7.5 | 1.99 | | 2.16 |
| 8.0 | | 1.7 | 2.48 |
| | Alanine | | |
| 6.0 | | 1.4 | 0.55 |
| 7.0 | | 1.6 | 1.36 |
| 7.5 | 1.76 | | 1.77 |
| 8.0 | | 2.1 | 2.11 |

[a] Matsuo's constants have been divided by the concentration of water (55.5 *M*) in keeping with our definition of $K_{cond}$: $K_{cond} = \Sigma_k[(PLP \cdot L)H_k]/\Sigma_i[PLPH_i]\Sigma_j[LH_j]$.

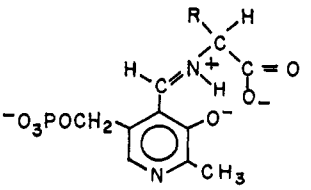
The glycinate and alaninate Schiff bases have very similar stabilities and proton binding constants, with the values for the latter amino acid being slightly lower in all cases. The first proton accepted by the tetraanionic Schiff base is most likely predominantly bound to the imine nitrogen judging from the spectral properties[8,23] of these species and the similarity of the $pK_{5a}$ values in Table I (11.88, G; 11.77, A) to that observed for N-salicylideneglycine (11.21). The second proton to be acquired by the PLP–Schiff bases ($pK_{4a}$ = 6.65, G; 6.33, A) is probably distributed appreciably between the phosphate group ($pK_{3a}$ = 5.98 for PLP) and the ring nitrogen. Metzler[24] gives 5.9 for the $pK_a$ for the protonation of the pyridine nitrogen in N-pyridoxylideneglycinate. The carboxylate group is likely involved to a negligible extent since N-salicylideneglycinate does not accept a proton in this pH region. Most likely both forms IV and V are present with IV predominating.



IV ⇌ V

The formation constants for the simple Zn(II)–PLP species are also given in Table I. From the values given, the proton ionization constants of $Zn(PLP)H_2^+$ are calculated to be $pK_{1a}$ = 5.1 and $pK_{2a}$ = 6.3. Owing to the high sensitivity of the phenol and pyridinium $pK_a$ values of hydroxypyridines to substituent and electrostatic effects, it is not possible from these values to unambiguously assign the binding of the metal ion to one or the other of these sites. A tentative mode of chelation may be proposed by considering additional evidence. The formation constant for $Zn^{2+} + PLP^{3-} \rightleftharpoons ZnPLP^-$ ($\log K = 3.6$) is greater than that for $Zn^{2+} + PL^- \rightleftharpoons ZnPL^+$ ($\log K = 2.3$), indicating some participation by the phosphate group in complex formation. The relatively high value for the formation constant indicates that chelation also involves other groups in the PLP molecule. Molecular models show that it is possible to bind a metal ion to the phenolate oxygen and aldehyde group, as with salicylaldehyde complexation, and still bring the phosphate group over to the vicinity of the metal ion. Although the resulting phosphate–metal ion distance is somewhat longer than normal, the reduction in electrostatic energy would serve to increase the stability of the complex. Such quasi-tridentate behavior by PLP would leave only the pyridine nitrogen available for accepting the first proton ($pK_a$ = 6.3) and reduce the tendency for the phosphate group to accept the second proton ($pK_a$ = 5.1).

The stabilities of the PLP–Schiff base complexes with Zn(II) are slightly higher than those found with salicylaldehyde and are much greater than those found for pyridoxal. The ligand enhancement factor (LEF = $\log \beta_{110} - \log \beta_{100} - \log \beta_{010}$) is a measure of the effect of the difference between the coordination of the Schiff base as opposed to the independent binding of the carbonyl compound and amino acid. The large values (LEF = 2.1 for alanine and 1.8 for glycine) indicate that by far the ligands in these ternary PLP complexes exist as the Schiff bases. The value of LEF (1.9) found for N-salicylideneglycinatozinc(II) is almost the same as that found for PLP. Since phosphate–metal interactions caused the value of $\beta_{100}$ to be enhanced in $ZnPLP^-$, these interactions must also be present in the Zn–PLP Schiff base complexes; otherwise



III

(23) O. Heinert and A. E. Martell, *J. Amer. Chem. Soc.*, 85, 188 (1963).

(24) D. E. Metzler, *ibid.*, 79, 485 (1957).

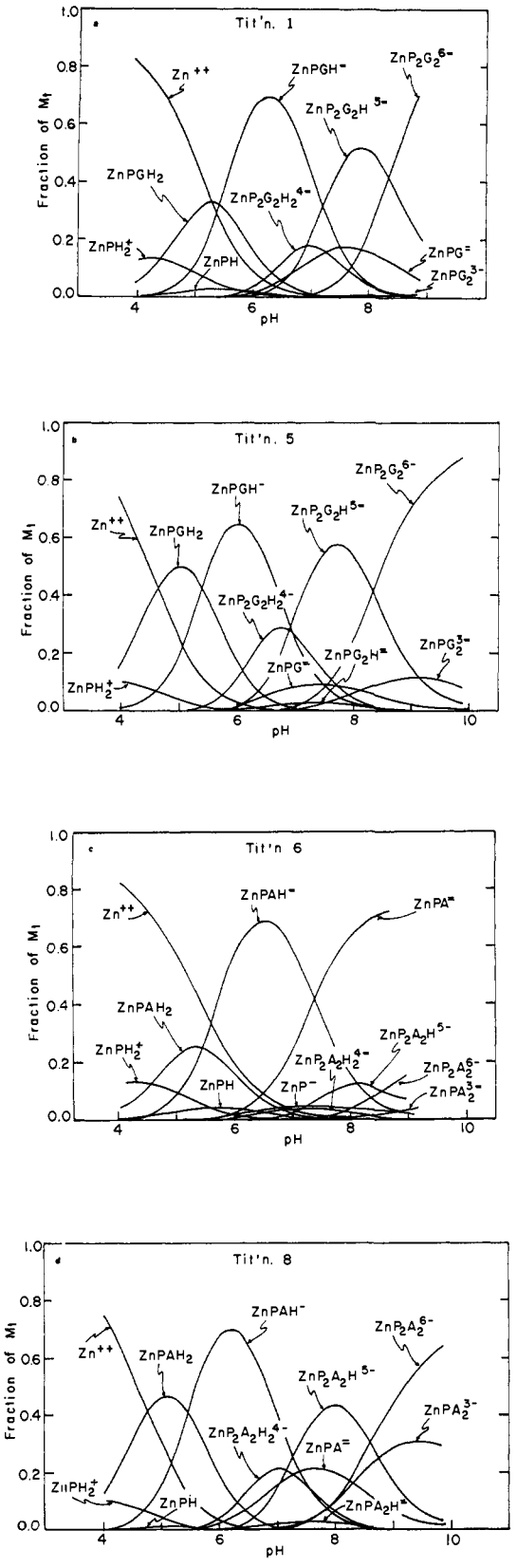


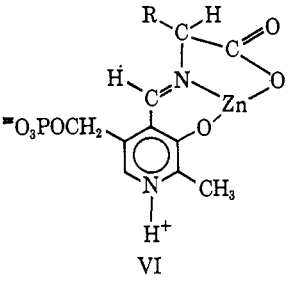

Figure 2. Distribution of species as a function of pH in some titrations of Figure 1. To conserve space, P = $PLP^{3-}$, G = glycinate, A = alaninate: (a) glycine titration no. 1, (b) glycine titration no. 5, (c) alanine titration no. 6, and (d) alanine titration no. 8.

the LEF for PLP would show a relative decrease. The principal factor determining LEF for salicylaldehyde appears simply to arise from the chelate effect which causes the binding of one tridentate ligand to be preferred to the binding of two bidentate ligands.[9] The PLP systems appear to be "normal" in this respect.

Further evidence for Schiff base formation, in addition to spectral changes, are the high stabilities of the $Zn(PLP)_2L_2^{6-}$ species. Zn(II) complexes having this large number of ligands would be very unstable in aqueous solutions in the absence of strong interligand interactions.

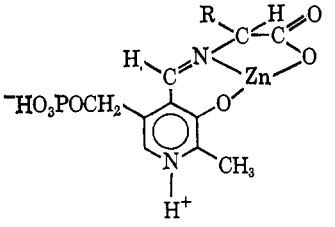
The steric effect of the methyl group of alanine seems to play no significant role in the relative stabilities of the Zn(II)–Schiff base complexes. $\beta_{110}$ and $\beta_{120}$ are essentially equal for the alanine and glycine cases, indicating that the two Schiff bases have about the same affinities for Zn(II) coordination. However, $\beta_{220}$ in the alanine case is about one-tenth as large as that for glycine. This decrease is attributed to a field effect caused by increasing the hydrocarbon content in the region of a highly charged 6− complex ion.

The addition of the first proton to $Zn(PLP\cdot L)^{2-}$ corresponds to $pK_a$ of 7.4 for both glycinate and alaninate. This value is practically identical with $pK_a$ of 7.3 found for the protonation of $Zn(PL\cdot G)$.[10,24] Since no phosphate protonation, but only pyridine nitrogen protonation, is possible in this latter case, it appears that nitrogen protonation also occurs with PLP to give VI.



VI

The second proton, $pK_a$ = 5.4, must therefore be added to the phosphate group to give VII.



VII

$Zn(PLP\cdot L)_2^{6-}$ has a high negative charge. In addition, the binding of a proton is aided statistically by the presence of two identical ligands. Therefore, it is not surprising that the $pK_a$ (8.3 to 8.6) of $Zn(PLP\cdot L)_2H^{5-}$ is higher than that (7.4) for $Zn(PLP\cdot L)H^-$. The $pK_a$ of the second proton bound to the bis complex lies in the range 6.9–7.2. After making a statistical correction of 0.3 $pK_a$ unit, assuming two equivalent protons, these values are seen to bracket the $pK_a$ for the ionization of the pyridinium nitrogen of the mono Schiff base complex, $Zn(PLP\cdot L)H^-$. Thus, it appears that both protons in $Zn(PLP\cdot L)_2H_2^{4-}$ are predominantly bound to pyridine nitrogen atoms. No higher protonated forms of these bis Schiff base complexes were observed in the present work because in lower pH

regions where phosphate protonation occurs, the simultaneous repression of the free ligand levels, also through protonation, causes the concentrations of the bis species to drop to negligible levels (see the distribution curves given in Figure 2). Similarly, the mono Schiff base complexes are unstable in the pH region where the phosphate group acquires a second proton (pH ~1.6), so $Zn(PLP \cdot L)H_3^+$ is not observed.

The ionization of the pyridinium group of pyridoxal shows interesting differences between the various species.[8,10] In pyridoxal itself the $pK_a$ of this group is 8.5 but drops to 5.9 in the protonated Schiff base complex, $PLGH_2$. The value rises to 7.3 when zinc replaces the imine proton to give $Zn(PL \cdot G)H^+$. Changes of a similar qualitative nature are expected for the pyridinium group in the PLP system. That is, the ionization of the pyridinium group of uncomplexed PLP would appear to lie at a value higher than 7.3 which is observed in the Schiff base complexes. Similar considerations can be applied to the ionization of the phosphate group. The metal ion–phosphate interactions, which appear to be present, would cause the $pK_a$ value of the phosphate group in the binary and ternary complexes to be lower than that observed for uncomplexed PLP. Thus, the phosphate group in uncomplexed PLP would appear to have a value higher than 5.5. On the basis of these assumptions, the order of ionization for uncomplexed PLP may be placed $pK_{1a}$ ($H_2PO_4^-$), $pK_{2a}$ (phenol), $pK_{3a}$ ($^-HPO_4^-$), and $pK_{4a}$ (pyridinium). This order places the ionization of the PLP phenol group ($pK_{2a}$ = 3.63) closer to the value ($pK_{1a}$ = 4.25)[8] assigned to the phenol group of PL and brings the ratio of the phosphate ionization constants more in line with the value of $10^5$ observed for phosphoric acid. This order is similar to that proposed by Anderson and Martell[22] with the exception of the interchange of the monohydrogen phosphate and phenol groups.

The distribution curves for zinc(II) in some of the titrations obtained here are given in Figure 2. It can be seen that protonated species predominate at pH 7 with the unprotonated forms becoming increasingly important as the pH increases slightly above neutrality. These distribution curves are very similar to those found for pyridoxal.[10] The chief differences between the two systems arise from the presence of $Zn(PLP)H_i^{i-1}$ ($i$ = 0, 1, 2) species, which become less important above pH 5, and $Zn(PLP \cdot L)H_2$, which becomes important below pH 6.5. Thus, there is not a marked increase in complexity of the systems when PLP–metal ion systems themselves are studied rather than seemingly simpler metal ion–PL systems. Considering PL hemiacetal formation, PLP may actually be preferable under many circumstances.

# Studies on Nitrogen-Coordinated Complexes. Preparations and Reactions of Hydrido–Phosphine Complexes of Ruthenium and Rhodium

**Takashi Ito, Shoji Kitazume, Akio Yamamoto, and Sakuji Ikeda**

*Contribution from the Research Laboratory of Resources Utilization, Tokyo Institute of Technology, Meguro, Tokyo, Japan.*
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**Abstract:** Tetrakis(triphenylphosphine)ruthenium dihydride, $H_2Ru(PPh_3)_4$, and tetrakis(triphenylphosphine)-rhodium hydride, $HRh(PPh_3)_4$, were synthesized by the reaction of $MCl_3$ or $M(acac)_3$ (M = Ru and Rh), triphenylphosphine, and $Et_3Al$ in THF. Both light yellow complexes were characterized by means of elemental analyses, thermal decompositions, infrared spectroscopy, and other chemical reactions. $H_2Ru(PPh_3)_4$ was found to dissociate in solution into free triphenylphosphine and $H_2Ru(PPh_3)_3$, and the reversible combination of $H_2Ru(PPh_3)_3$ with $N_2$ and $H_2$ was demonstrated. In the exchange reaction with deuterium, 24–25 hydrogens per mole of the ruthenium complex were found to participate, indicating the reversible exchange of all the *ortho* hydrogens of phenyl groups with deuterium, whereas in the rhodium complex, the number of exchangeable hydrogens was about seven to nine. The reactions of the ruthenium complex with TCNE gave $(TCNE)_3Ru(PPh_3)_2$, whereas acrylonitrile was polymerized to high polymer by the complex.

The study of the nitrogen-coordinated transition metal complexes is now becoming one of the most attractive subjects with special reference to the mechanistic investigation of ammonia producing reactions by the systems composed of transition metal compounds and reducing agents[1] or by biological systems.[2]

A variety of transition metal systems which can combine with molecular nitrogen through reactions with organic nitrogen compounds[3–8] or with atmospheric nitrogen[9–19] have been reported.

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(2) See, for example, R. W. F. Hardy and R. C. Burns, *Ann. Rev. Biochem.*, **37**, 331 (1968).

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